



UNIVERSITI PUTRA MALAYSIA

**ENZYMATIC SYNTHESIS OF OLEYL OLEATE, A LIQUID WAX
ESTER, IN A STIRRED TANK REACTOR**

SALINA MAT RADZI.

FS 2006 36

**ENZYMATIC SYNTHESIS OF OLEYL OLEATE, A LIQUID WAX
ESTER, IN A STIRRED TANK REACTOR**

By

SALINA BINTI MAT RADZI

**Thesis Submitted to the School of Graduate Studies, Universiti
Putra Malaysia, in Fulfilment of the Requirement for the Degree of
Doctor of Philosophy**

June 2006



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ENZYMATIC SYNTHESIS OF OLEYL OLEATE, A LIQUID WAX ESTER, IN A STIRRED TANK REACTOR

By

SALINA BINTI MAT RADZI

June 2006

Chairman : Professor Hjh. Mahiran Basri, PhD

Faculty : Science

High performance enzymatic synthesis of oleyl oleate, a liquid wax ester was successfully synthesized via enzymatic esterification reaction of oleic acid and oley alcohol. Immobilised *Candida antartica* lipase B (Novozym 435) was used as biocatalyst. The study was divided into four parts which are the optimisation of reaction synthesis at different scales, the reactor study in term of mixing efficiency, the stability of immobilised enzyme and the analysis and characterization of the product of the reaction.

Preliminary synthesis of oleyl oleate was carried out in a small scale reaction with a total volume of 3.5 mL using screw-capped vials. Optimisation reaction study via conventional method of varying one parameter at-a-time approach was carried out. A high percentage

conversion yield of >90% was achieved at optimum reaction time of 5 min, reaction temperature of 40-60°C, molar ratio of substrates (oleyl alcohol/oleic acid) of 2:1, amount of enzyme of 0.4 g and organic solvents of Log P \geq 3.5 at fixed agitation speed of 150 rpm.

Investigation in larger scale production of oleyl oleate was performed using 2 L stirred tank reactor (STR). The reaction was scaled-up to 300X with a total volume of 1.05 L. A high percentage conversion of oleyl oleate was achieved of >95% by conventional experiment method at reaction time of 30 min, agitation speed of 400 rpm, reaction temperature of 45-50°C, molar ratio of substrate (oleyl alcohol/oleic acid) of 2:1 and amount of enzyme of 90 g.

The reaction synthesis was further optimised by response surface method (RSM) based on five-level, three-variable central composite rotatable design (CCRD) to evaluate the interactive effects of important parameters in larger scale processing. Generally, simultaneously increasing amount of enzyme, agitation speed and reaction temperature would improved the yields. A high percentage conversion of 97.4% was achieved under the optimum condition, which compared well with the maximum predicted value of 97.7%.

In order to improve the production and productivity of oleyl oleate to the highest amount that can be produced in a 2 L STR, the reaction was

synthesized in a solvent-free system. Maximum scaling-up of substrate concentration that can be achieved in the reactor vessel was 900X as compared to 300X previously. The production and productivity of oleyl oleate were successfully improved from 295.39 g/L/h to 705.76 g/L/h and 310.16 g/h to 952.78 g/h, respectively.

Reactor study on the performance of 2 L STR as a mixing device was evaluated to improve the mixing efficiency. The rheological property of the reaction mixture exhibited Newtonian behaviour. Rushton turbine impeller showed better performance in degree of mixing, as compared to AL Hydrofoil impeller whereby a high Reynolds number of $>10^4$ was achieved at 400 rpm, which exhibit a turbulent flow pattern. There was significant effect to the mixing improvement on the enzyme particles distribution by using a 2 impellers system with spacing of 30 mm.

The enzyme showed high stability against heat as shown by the high percentage conversion of wax ester. Novozym 435 retained its synthetic activity up to 9 uses and 4 uses in screw-capped vials and STR, respectively. The effect of shear forces due to the mechanical agitation speed on the enzyme morphology was determined by scanning electron microscope (SEM). Although small rupture on the surface of enzyme was observed when increasing the agitation speed, the enzyme activity was very high even at high agitation speed.

Analysis of product was evaluated by spectroscopy method of Fourier transform-infrared spectroscopy (FT-IR) and gas chromatography-mass spectroscopy (GC-MS) to identify the product obtained. Characteristics of oleyl oleate were also examined, which include iodine value, saponification value, acid value and ester value. Solubility of oleyl oleate in methanol and ethanol was comparatively lower as compared to the solubility at higher chain length of alcohols. This compound seems compatible in most of oils and stable even after heating up to 90°C and overnight storage at room temperature.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**SINTESIS ENZIM BAGI OLEIL OLEAT, CECAIR ESTER LILIN, DI
DALAM TANGKI REAKTOR BERGERAK**

Oleh

SALINA BINTI MAT RADZI

Jun 2006

Pengerusi : Profesor Hjh. Mahiran Basri, PhD

Fakulti : Sains

Sintesis enzim berprestasi tinggi bagi oleil oleat, cecair ester lilin telah disintesis dengan jayanya melalui tindakbalas esterifikasi enzim oleh asid oleik dan alkohol oleil. Lipase B *Candida antartica* tersekatgerak (Novozim 435) telah digunakan sebagai biopemangkin dalam tindakbalas ini. Kajian ini telah dibahagikan kepada empat bahagian iaitu pengoptimuman sintesis tindakbalas pada skala berbeza, kajian reaktor berhubung dengan keefisienan percampuran, kestabilan enzim tersekatgerak dan analisis dan pencirian produk tindakbalas.

Permulaan sintesis tindakbalas bagi oleil oleat telah dijalankan pada skala kecil dengan jumlah isipadu adalah 3.5 mL menggunakan botol kecil bertutup-skru. Kajian pengoptimuman tindakbalas melalui kaedah

konvensional iaitu memvariasikan satu parameter pada-satu-masa telah dijalankan. Peratusan penukaran hasil yang tinggi iaitu >90% telah dicapai pada masa tindakbalas optimum iaitu 5 min, suhu tindakbalas pada 40-60°C, nisbah molar bahan tindakbalas (alkohol oleil/ asid oleik) pada 2:1, amaun enzim pada 0.4 g dan pelarut organik pada log $P \geq 3.5$ dengan kelajuan putaran yang ditetapkan pada 150 rpm.

Penyelidikan pada pengeluaran skala lebih besar bagi oleil oleat telah dihasilkan dengan menggunakan 2 L tangki reaktor bergerak (STR). Skala tindakbalas telah ditingkatkan kepada 300X dengan jumlah isipadu pada 1.05 L. Peratusan penukaran oleil oleat yang tinggi telah dicapai pada >95% dari kaedah eksperimen secara konvensional pada masa tindakbalas pada 30 min, kelajuan putaran pada 400 rpm, suhu tindakbalas pada 45-50°C, nisbah molar bahan tindakbalas (alkohol oleil/asid oleik) pada 2:1 dan amaun enzim sebanyak 90 g.

Sintesis tindakbalas telah dioptimumkan selanjutnya dengan kaedah permukaan respon (RSM) berdasarkan lima-peringkat, tiga-pembolehubah rekaan pusat komposit berputar (CCRD) untuk menilai kesan interaktif bagi parameter-parameter yang penting di dalam pemprosesan pada skala lebih besar. Secara amnya, dengan peningkatan secara serentak bagi amaun enzim, kelajuan putaran dan suhu tindakbalas akan meningkatkan hasil. Peratusan penukaran yang

tinggi iaitu 97.4% diperolehi di bawah keadaan optimum, di mana ia telah dibandingkan dengan baik dengan nilai anggaran maksimum iaitu 97.7%. Bagi meningkatkan pengeluaran dan produktiviti oleil oleat kepada amaun yang paling tinggi yang dapat dihasilkan dalam 2 L STR, tindakbalas telah disintesis di dalam sistem tanpa pelarut. Peningkatan skala bahan tindakbalas yang maksimum dapat dihasilkan di dalam bekas reaktor adalah 900X berbanding 300X sebelumnya. Pengeluaran dan produktiviti oleil oleat telah ditingkatkan masing-masing dengan jayanya daripada 295.39 g/L/j kepada 705.76 g/L/j dan 310.16 g/j kepada 952.78 g/j.

Kajian reaktor ke atas prestasi 2 L STR sebagai peralatan percampuran telah dilakukan untuk meningkatkan keefisienan percampuran. Sifat riologi campuran tindakbalas telah menunjukkan sifat Newtonian. Penggerak turbin Rushton telah menunjukkan prestasi terbaik di dalam percampuran, berbanding dengan penggerak AL Hidrofoil, di mana nombor Reynolds yang tinggi $>10^4$ telah dicapai pada 400 rpm, di mana ia menunjukkan gerakan aliran bergelora. Terdapat kesan yang baik pada keefisienan percampuran dengan menggunakan sistem 2 penggerak dengan jarak 30 mm.

Enzim menunjukkan kestabilan yang tinggi terhadap haba dengan peratusan penukaran ester lilin yang tinggi. Aktiviti sintetik Novozim 435 kekal masing-masing, sehingga 9 kali penggunaan dan 4 kali penggunaan

di dalam botol kecil bertutup-skru dan STR. Kesan tekanan daripada kelajuan putaran mekanikal terhadap morfologi enzim telah ditentukan dengan pengimbas mikroskop electron (SEM). Walaupun rekahan kecil pada permukaan enzim telah didapati semasa kelajuan putaran ditingkatkan, aktiviti enzim adalah sangat tinggi walaupun pada kelajuan putaran yang tinggi.

Analisis produk telah dilakukan dengan kaedah spektroskopi iaitu spektroskopi inframerah (FT-IR) dan spektroskopi jisim-kromatografi gas (GC-MS) untuk mengenalpasti produk yang telah di perolehi. Ciri-ciri oleil oleat juga diperiksa, termasuk nilai iodin, nilai saponifikasi, nilai asid dan nilai ester. Kelarutan oleil oleat dalam metanol dan etanol adalah lebih rendah berbanding dengan kelarutan dalam alkohol berantai panjang. Kompaun ini di dapati sesuai di dalam kebanyakan minyak dan stabil walaupun selepas dipanaskan sehingga 90°C dan disimpan sepanjang malam pada suhu bilik.

ACKNOWLEDGEMENTS

Alhamdulillah, all praises to Allah S.W.T., for giving me the strength to endure all challenges and complete this study.

My sincere appreciation goes out to Prof. Dr. Hjh. Mahiran Basri, Prof. Dr. Abu Bakar Salleh, Prof. Dr. Arbakariya Ariff, Dr. Rosfarizan Mohamad and other members in Enzyme and Microbial Technology Research Group for their great concern, advice, patience, persistent encouragement and invaluable assistance from the beginning till the end of this study.

A special thank to Mr. Rizal Kapri, Mr. Sobri Mohd. Akhir and members of the Fermentation and Enzyme Technology Laboratory, Institute of Bioscience, whose help and cooperation were invaluable during the course of the study.

I am grateful to staff of Microscopy and Microanalysis Unit, Institute of Bioscience especially to Assoc. Prof. Dr. Fauziah Othman and Mr. Safari for their help in the progress of this study.

Love and thanks to my family for their understanding, constant supports and simply being here, loving me with all their hearts.

A special thank also goes to Kak Erin, Salhah, Pei Sin, Ita, Yamin, Yati and other members of Lab 401, without whom life would be a bore and uneventful. Thank you for the bond of friendship and for making my stay in UPM a bearable one with many sweet memories and experiences. Thank you for being friends in need.

Finally, thank you to the Ministry of Science, Technology and Environment and Universiti Putra Malaysia for their financial support and facilities.

I certify that an Examination Committee has met on 8th June 2006 to conduct the final examination of Salina Binti Mat Radzi on her Doctor of Philosophy thesis entitled "Enzymatic Synthesis of Oleyl Oleate, a Liquid Wax Ester, in a Stirred Tank Reactor" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

MOHAMAD BASYARUDDIN ABDUL RAHMAN, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

FAUJAN AHMAD, PhD


Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

SURAINI ABDUL AZIZ, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

MOHAMAD ROJI SARMIDI, PhD

Professor
Faculty of Chemical Engineering and Natural Resources
Universiti Teknologi Malaysia
(External Examiner)



HASANAH MOHD GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: **11 JUL 2006**

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

MAHIRAN BASRI, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

ABU BAKAR SALLEH, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

ARBAKARIYA ARIFF, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

ROSFARIZAN MOHAMAD, PhD

Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)



AINI IDERIS, PhD

Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 AUG 2006

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



SALINA BINTI MAT RADZI

Date: 21 /6 /2006

TABLE OF CONTENTS

	PAGE
ABSTRACT	ii
ABSTRAK	vi
ACKNOWLEDGEMENTS	x
APPROVAL	xii
DECLARATION	xiv
LIST OF TABLES	xvii
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxv
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	6
2.1 History and Diversity of Waxes	6
2.2 Wax Ester	8
2.2.1 Application of Wax Ester	9
2.3 Jojoba Oil	10
2.4 Synthetic Wax Ester	12
2.4.1 Chemical Synthesis	13
2.4.2 Enzymatic Synthesis	14
2.5 Commercial Synthesis of Wax Ester for Industrial Application	17
2.6 Lipase as Biocatalyst	22
2.6.1 Immobilised Lipase	26
2.6.2 Enzyme in Organic Synthesis	28
2.6.3 The Use of Lipase in Ester Synthesis	29
2.7 Response Surface Methodology (RSM)	30
2.7.1 The Four Steps in RSM	31
2.7.2 The Uses of RSM	33
2.7.3 Application of RSM to the Enzymatic Synthesis	34
2.8 Bioprocess Development	35
2.8.1 Steps in Bioprocess Development	37
2.9 Fluid Flow and Mixing	39
2.9.1 Classification of Fluids	39
2.9.2 Viscosity	40
2.9.3 Mixing	44
2.10 Enzyme Reactor Operation	53
2.10.1 Stirred Tank	57



2.10.2	Enzymatic Synthesis of Ester using a Reactor System	59
2.11	Summary	60
3	MATERIALS AND METHODS	64
3.1	Materials	63
3.2	Methods	65
3.2.1	Experimental Design	66
3.2.2	Enzymatic Synthesis of Oleyl Oleate	67
3.2.3	Optimisation of Reaction Synthesis	69
3.2.4	Reactor Study	84
3.2.5	Stability of Immobilised Enzyme	89
3.2.6	Analysis and Characterization of Oleyl Oleate	92
4	RESULTS AND DISCUSSION	98
4.1	Enzymatic Synthesis of Oleyl Oleate	98
4.2	Optimisation of Reaction Synthesis	100
4.2.1	Reaction in Screw-Capped Vial	100
4.2.2	Reaction in 2 L Stirred Tank Reactor	115
4.2.3	Response Surface Methodology (RSM)	127
4.2.4	Improvement of Oleyl Oleate Production and Productivity by Manipulating Amount of Solvent and Reaction Substrate in 2 L STR	143
4.2.5	Summary	150
4.3	Reactor Study	154
4.3.1	Effect of Mixing on the Reactor Performance	154
4.3.2	Reactor Modification In Term of Impeller Design	171
4.3.3	Summary	177
4.4	Stability of Immobilised Enzyme	180
4.4.1	Effect of Heat on Enzyme on Enzyme Stability	180
4.4.2	Reusability of Enzyme in Screw-Capped Vial	183
4.4.3	Reusability of Enzyme in 2 L Stirred Tank Reactor	186
4.4.4	Effect of Varying Agitation Speed on the Surface Morphology of the Immobilised Enzyme Particle	188
4.4.5	Summary	203
4.5	Analysis and Characterization of Oleyl Oleate	204
4.5.1	Isolation and Purification of Oleyl Oleate	204
4.5.2	Identification of Oleyl Oleate	204
4.5.3	Characterization of Oleyl Oleate	212
4.5.4	Summary	219
5	CONCLUSION	220
5.1	Recommendations for Further Studies	223
	REFERENCES	225
	APPENDICES	238
	BIODATA OF THE AUTHOR	274

LIST OF TABLES

Table		Page
1	Application of Wax Esters	9
2	Catalytic System of Producing a Synthetic Wax Ester	12
3	Comparison Between Catalytic Systems for Wax Ester Production	13
4	Industrial Applications of Microbial Lipases	25
5	Major Products of Biological Processing	36
6	Common Non-Newtonian Fluids	43
7	Viscosity Ranges for Different Impellers	48
8	Weight of Oleyl Alcohol and Oleic Acid for the Study on the Synthesis of Oleyl Oleate in Screw-Capped Vial	72
9	List of Solvents and Their Log P Values	73
10	Weight of Oleyl Alcohol and Oleic Acid for the Study on the Synthesis of Oleyl Oleate in 2 L Stirred Tank Reactor	76
11	Summary of Experimental Design of RSM	79
12	Design Matrix of the Actual and Coded Levels for the Three-Factor Central Composite Rotatable Design (CCRD)	80
13	Optimal Conditions Derived by RSM (Quadratic Model)	82
14	The Design Matrix of the Actual Experiments Carried Out for Developing the Model	128
15	ANOVA for Synthesis of Oleyl Oleate, (Quadratic Model)	129
16	R-Squared (R^2) Analysis of Quadratic Model	129



17	Values of Significance of Regression Coefficients for Synthesis of Oleyl Oleate (Quadratic Model)	131
18	Optimal Conditions Derived by RSM (Quadratic Model)	142
19	Summary of Optimisation Study on Reaction Synthesis	153
20	Reynolds Number and Fluid Flow Pattern using Rushton Turbine Impeller	170
21	Reynolds Number and Fluid Flow Pattern using AL Hydrofoil Impeller.	170
22	Summary of Reactor Study in Term of agitation System	180
23	Properties of Oleyl Oleate	214
24	Compatibility of Oleyl Oleate in Various Type of Cosmetic Oils	218

LIST OF FIGURES

Figure		Page
1	Raw Materials Used in Large Scale Production of Ester	18
2	Large Scale Production of Oleyl Oleate by Chemical Esterification Reaction	20
3	Large Scale Production of Oleyl Oleate by Enzymatic Esterification Reaction	20
4	Large Scale Production of Glycerol Monooleate by Enzymatic Transesterification Reaction	21
5	Large Scale Production of Glycerol Monooleate by Chemical Transesterification Reaction	22
6	Flow Curve for Newtonian Fluids	41
7	Flow Curve for Non-Newtonian Fluids	42
8	Typical Configuration of a Stirred Tank	46
9	Impeller Designs	47
10	Flow Pattern Produced by a Radial Flow Impeller in a Baffled Tank	49
11	Flow Pattern Produced by a Axial Flow Impeller in a Baffled Tank	50
12	Correlation Between Power Number and Reynolds Number for Rushton Turbine Impeller in Baffled Tank	52
13	Reactor Configurations	56
14	Typical Schematic Diagram of Stirred Tank Reactor	58
15	Flow Diagram of Experimental Design	66

16	Effect of Reaction Time on the Enzymatic Synthesis of Oleyl Oleate in Screw-Capped Vial (3.5 mL)	101
17	Effect of Reaction Temperature on the Enzymatic Synthesis of Oleyl Oleate in Screw-Capped Vial (3.5 mL)	104
18	Effect of Molar Ratio of Substrates on the Enzymatic Synthesis of Oleyl Oleate in Screw-Capped Vial (3.5 mL)	107
19	Effect of Various Organic Solvents on the Enzymatic Synthesis of Oleyl Oleate in Screw-Capped Vial (3.5 mL)	110
20	Effect of Amount of Enzyme on the Enzymatic Synthesis of Oleyl Oleate in Screw-Capped Vial (3.5 mL)	113
21	Effect of Reaction Time on Enzymatic Synthesis of Oleyl Oleate in Stirred Tank Reactor (1.05 L)	116
22	Effect of Reaction Temperature on the Enzymatic Synthesis of Oleyl Oleate in Stirred Tank Reactor (1.05 L)	119
23	Effect of Molar Ratio of Substrate on the Enzymatic Synthesis of Oleyl Oleate in Stirred Tank Reactor (1.05 L)	122
24	Effect of Amount of Enzyme on the Enzymatic Synthesis of Oleyl Oleate in Stirred Tank Reactor (1.05 L)	124
25	Effect of Agitation Speed on the Enzymatic Synthesis of Oleyl Oleate in Stirred Tank Reactor (1.05 L)	126
26	Response Surface Plot of Agitation Speed versus Amount of Enzyme (BA) in Quadratic Model	133
27	Response Surface Plot of Reaction Temperature versus Amount of Enzyme (CA)	135

28	Response Surface Plot of Reaction Temperature versus Agitation Speed (CB)	137
29	Contour Plot of Agitation Speed versus Amount of Enzyme (BA)	139
30	Contour Plot of Reaction Temperature versus Amount of Enzyme (CA)	140
31	Contour Plot of Agitation Speed versus Reaction Temperature (BC)	141
32	Effect of Reducing the Amount of Solvent on the Percentage Conversion and Production of Oleyl Oleate in 2 L Stirred Tank Reactor	145
33	Effect of using High Substrate Concentration on the Percentage Conversion and Productivity of Oleyl Oleate in 2 L Stirred Tank Reactor	148
34	Effect of Agitation Speed on the Percentage Conversion of Oleyl Oleate using Rushton Turbine Impeller in Stirred Tank Reactor (1.05 L)	156
35	Effect of Agitation Speed on the Percentage Conversion of Oleyl Oleate using AL Hydrofoil Impeller in Stirred Tank Reactor (1.05 L)	157
36	Viscosity of Reaction Mixture at Different Agitation Speeds on Oleyl Oleate Production using Rushton Turbine Impeller	160
37	Viscosity of Reaction Mixture at Different Agitation Speeds on Oleyl Oleate Production using AL Hydrofoil Impeller	161
38	Flow Curve of Newtonian Fluid by using Rushton Turbine Impeller	164
39	Flow Curve of Newtonian Fluid by using AL Hydrofoil Impeller	165
40	Effect of Varying Shear Rate on the Viscosity of Fluid using Rushton Turbine Impeller	166

41	Effect of Varying Shear Rate on the Viscosity of Fluid using AL Hydrofoil Impeller	167
42	Effect of Number of Impeller	172
43	Effect of Impeller Spacing	175
44	Effect of Heat on Enzyme Stability	181
45	Reusability of Enzyme in Screw-Capped Vial	184
46	Reusability of Enzyme in Stirred Tank Reactor	187
47	Scanning Electron Microscope of Fresh Novozym 435 (50X Magnification)	191
48	Scanning Electron Microscope of Novozym 435 at 100 rpm using a Rushton Turbine Impeller (50X Magnification)	191
49	Scanning Electron Microscope of Novozym 435 at 100 rpm using a Rushton Turbine Impeller (3000X Magnification)	192
50	Scanning Electron Microscope of Novozym 435 at 200 rpm using a Rushton Turbine Impeller (50X Magnification)	192
51	Scanning Electron Microscope of Novozym 435 at 200 rpm using a Rushton Turbine Impeller (300X Magnification)	193
52	Scanning Electron Microscope of Novozym 435 at 200 rpm using a Rushton Turbine Impeller (3000X Magnification)	193
53	Scanning Electron Microscope of Novozym 435 at 300 rpm using a Rushton Turbine Impeller (50X Magnification)	194
54	Scanning Electron Microscope of Novozym 435 at 300 rpm using a Rushton Turbine Impeller (300X Magnification)	194

55	Scanning Electron Microscope of Novozym 435 at 300 rpm using a Rushton Turbine Impeller (3000X Magnification)	195
56	Scanning Electron Microscope of Novozym 435 at 400 rpm using a Rushton Turbine Impeller (50X Magnification)	195
57	Scanning Electron Microscope of Novozym 435 at 400 rpm using a Rushton Turbine Impeller (300X Magnification)	196
58	Scanning Electron Microscope of Novozym 435 at 400 rpm using a Rushton Turbine Impeller (3000X Magnification).	196
59	Scanning Electron Microscope of Novozym 435 at 100 rpm using a AL Hydrofoil Impeller (50X Magnification).	197
60	Scanning Electron Microscope of Novozym 435 at 100 rpm using a AL Hydrofoil Impeller (300X Magnification).	197
61	Scanning Electron Microscope of Novozym 435 at 100 rpm using a AL Hydrofoil Impeller (3000X Magnification).	198
62	Scanning Electron Microscope of Novozym 435 at 200 rpm using a AL Hydrofoil Impeller (50X Magnification)	198
63	Scanning Electron Microscope of Novozym 435 at 200 rpm using a AL Hydrofoil Impeller (300X Magnification)	199
64	Scanning Electron Microscope of Novozym 435 at 200 rpm using a AL Hydrofoil Impeller (3000X Magnification)	199
65	Scanning Electron Microscope of Novozym 435 at 300 rpm using a AL Hydrofoil Impeller (50X Magnification)	200

66	Scanning Electron Microscope of Novozym 435 at 300 rpm using a AL Hydrofoil Impeller (300X Magnification)	200
67	Scanning Electron Microscope of Novozym 435 at 300 rpm using a AL Hydrofoil Impeller (3000X Magnification)	201
68	Scanning Electron Microscope of Novozym 435 at 400 rpm using a AL Hydrofoil Impeller (50X Magnification)	201
69	Scanning Electron Microscope of Novozym 435 at 400 rpm using a AL Hydrofoil Impeller (300X Magnification)	202
70	Scanning Electron Microscope of Novozym 435 at 400 rpm using a AL Hydrofoil Impeller (3000X Magnification)	202
71	IR Spectrum of Oleic Acid	206
72	IR Spectrum of Oleyl Alcohol	207
73	IR Spectrum of Oleyl Oleate	208
74	Gas Chromatography Spectrum of Oleyl Oleate	210
75	Mass Spectroscopy Spectrum of Oleyl Oleate	211
76	General MS Fragmentation Pattern	211
77	Solubility of Oleyl Oleate in Various Chain-Length of Alcohol	216